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(21) International Application Number: PCT/US99/08850 (22) International Filing Date: 23 April 1999 (23.04.99) (30) Priority Data: 60/082,964 24 April 1998 (24.04.98) US (71) Applicant (for all designated States except US): WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): RICE, Charles, M. [US/US]; 7316 Colgate Avenue, University City, MO 63130 (US). FROLOV, Ilya [-/US]; St. Louis, MO (US). McBRIDE, M., Scott [-/US]; Madison, WI (US). (74) Agents: HOLLAND, Donald, R. et al.; Howell & Haferkamp, L.C., Suite 1400, 7733 Forsyth Boulevard, St. Louis, MO 63105-1817 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: CHIMERAS OF HEPATITIS C VIRUS AND BOVINE VIRAL DIARRHEA VIRUS			
(57) Abstract Disclosed is a polynucleotide comprising a chimeric viral RNA which contains: a 5' nontranslated region (5' NTR), an open reading frame (ORF) region, and a 3' nontranslated region (3' NTR) wherein at least one of said regions is chimeric. The chimeric region comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence. The chimeric viral RNA is replication-competent. Preferably the pestivirus sequence is from a bovine viral diarrhea virus and the heterologous nucleotide sequence is from a hepatitis C virus. Also disclosed are a method for identifying compounds having antiviral activity against hepatitis C virus, a genetically-engineered chimeric RNA virus and a vaccine against bovine viral diarrhea virus.			

Jumbo-1st pg + Claims ONLY

What is Claimed is:

1. A polynucleotide comprising a chimeric viral RNA which comprises:
 - (a) a 5' nontranslated region (5' NTR);
 - (b) an open reading frame (ORF) region; and
 - 5 (c) a 3' nontranslated region (3' NTR);wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein said chimeric viral RNA is replication-competent.
- 10 2. The polynucleotide of claim 1, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).
3. The polynucleotide of claim 2, wherein the BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAU.
- 15 4. The polynucleotide of claim 3, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).
5. The polynucleotide of claim 4, wherein the ORF and the 3' NTR consist of
- 20 second and third BVDV sequences.
6. The polynucleotide of claim 5, wherein the 5' terminal sequence comprises 5' GUAU.
- 25 7. The polynucleotide of claim 4, wherein the ORF comprises a second HCV sequence encoding at least one structural protein operably linked to a second BVDV sequence.
8. The polynucleotide of claim 1, wherein the pestivirus is BVDV and the
- 30 chimeric region is the 3' NTR.
9. The polynucleotide of claim 8, wherein the first HCV sequence in the chimeric 3' NTR comprises the HCV 98 bp 3' terminal element (SEQ ID NO:X) operably linked to the first BVDV sequence.

10. A method for identifying compounds having antiviral activity against hepatitis C virus (HCV) comprising the steps of:

(a) providing a first cell containing a chimeric viral RNA which is replication-competent in the cell, the chimeric viral nucleic acid comprising a 5' nontranslated region (5' NTR), an open reading frame (ORF) region; and a 3' nontranslated region (3' NTR);
5 wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV);

(b) providing a second cell containing the pestivirus; and

10 (c) comparing the replication efficiency of the chimeric viral RNA acid in the presence and absence of a test compound to the replication efficiency of the pestivirus in the presence and absence of the test compound,
wherein a greater reduction in compound-induced replication efficiency of the chimeric viral RNA than the pestivirus indicates the compound has anti-HCV activity.

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11. The method of claim 10, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

12. The method of claim 11, wherein the BVDV nucleotide sequence is located
20 at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAAU.

13. The method of claim 12, wherein the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

25 14. The method of claim 13, wherein the ORF and the 3' NTR comprise second and third sequences from the BVDV.

15. The method of claim 10, wherein the pestivirus is BVDV and the chimeric region is the 3' NTR.

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16. A genetically-engineered virus comprising a chimeric RNA genome which comprises:

(a) a 5' nontranslated region (5' NTR);

(b) an open reading frame (ORF) region; and

35 (c) a 3' nontranslated region (3' NTR);

wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein said chimeric RNA genome is replication-competent.

5 17. The genetically-engineered virus of claim 16, wherein the chimeric region is the 5' NTR and the first pestivirus nucleotide sequence is from a bovine viral diarrhea virus (BVDV).

 18. The genetically-engineered virus of claim 16, wherein the BVDV nucleotide
10 sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

 19. A vaccine against bovine viral diarrhea virus (BVDV) comprising an
15 immunogenically-effective amount of a genetically-engineered virus comprising a chimeric RNA genome having:

- (a) a 5' nontranslated region (5' NTR);
- (b) an open reading frame (ORF) region; and
- (c) a 3' nontranslated region (3' NTR);

20 wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from BVDV in operable linkage with a first nucleotide sequence from an hepatitis C virus (HCV), and wherein the genetically-engineered virus is attenuated as compared to BVDV.

 20. The vaccine of claim 19, wherein the chimeric region is the 5' NTR and the
25 BVDV nucleotide sequence is located at the 5' terminus of the chimeric 5' NTR and comprises 5' RUAAU and the first HCV nucleotide sequence in the chimeric 5' NTR comprises an internal ribosome entry site (IRES).

 21. A polynucleotide comprising a chimeric viral RNA which comprises:

- 30 (a) a 5' nontranslated region (5' NTR);
- (b) an open reading frame (ORF) region; and
- (c) a 3' nontranslated region (3' NTR);

wherein at least one of said regions is chimeric and comprises a first nucleotide sequence from a pestivirus in operable linkage with a heterologous nucleotide sequence and wherein
35 said chimeric viral RNA is replication-competent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08850

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/29, 39/295; C12Q 1/70; C12N 7/01; C07H 21/02

US CL : 424/218.1, 228.1; 435/5, 235.1; 536/23.72

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/218.1, 228.1; 435/5, 235.1; 536/23.72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; Derwent/WEST; DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	FROLOV et al. cis-acting RNA elements required for replication of bovine viral diarrhea virus-hepatitis C virus 5' nontranslated region chimeras. RNA. November 1998, Vol. 4, pages 1418-1435, see entire document.	1-8, 10-21
Y,P	MALET et al. Yellow fever 5' noncoding region as a potential element to improve hepatitis C virus production through modification of translational control. Biochem. Biophys. Res. Commun. 18 December 1998, Vol. 253, No. 2, pages 257-264, see entire document.	1-8, 10-21

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 JULY 1999

Date of mailing of the international search report

10 SEP 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08850

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LU et al. Poliovirus chimeras replicating under the translational control of genetic elements of hepatitis C virus reveal unusual properties of the internal ribosomal entry site of hepatitis C virus. Proc. Natl. Acad. Sci. USA. 20 February 1996, Vol. 93, No. 4, pages 1412-1417, see entire document.	1-8, 10-21
Y	VASSILEV et al. Authentic and chimeric full-length genomic cDNA clones of bovine viral diarrhea virus that yield infectious transcripts. J. Virol. January 1997, Vol. 71, No. 1, pages 471-478, see entire document.	1-8, 10-21
Y	VENUGOPAL et al. Towards a new generation of flavivirus vaccines. Vaccines. 1994, Vol. 12, No. 11, pages 966-975, see entire document.	1-8, 10-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08850

Box I. Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

CLAIM 9 RECITES "SEQ ID NO:X" WHICH EXPRESSION IS NOT UNDERSTOOD.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II. Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.